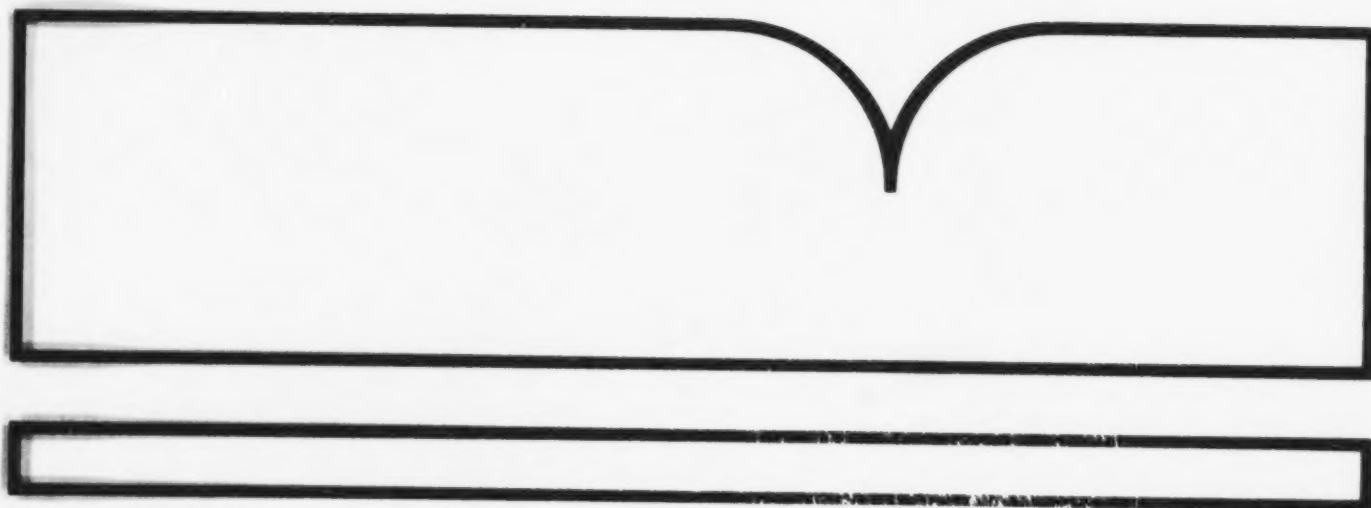


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Use of Molecular Reactivity
Criteria to Predict Toxicity of Xenobiotics

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THE USE OF MOLECULAR REACTIVITY CRITERIA TO PREDICT TOXICITY OF XENOBIOTICS

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ABSTRACT

The physicochemical basis for mechanisms of action of toxic xenobiotics is discussed in reference to theoretical methods that can be used to understand the relation of toxicity to the structure of these chemicals. Molecular properties that determine the chemical reactivity of the compounds are proposed as parameters in the analysis of such structure-activity relationships and as criteria for predicting potential toxicity. Special attention is given to quantitative methods for structural superposition of molecules and for superposition of their reactivity characteristics, as well as to the quantum mechanical methods for the calculation of molecular properties that can serve as such reactivity criteria. These approaches are illustrated by an application to the comparison between the properties of pyridine, which is toxic, and its 2,6-dimethyl derivative which appears to be devoid of similar toxic properties.

INTRODUCTION

The analysis of molecular determinants for the toxic actions of xenobiotics is encumbered by their enormous chemical variety and by the combination of specific and non-specific processes that can lead to toxicity [1].

A common approach in the evaluation of the actions of such agents is the assay of their effects on receptors and enzyme systems on which they often mimic endogenous substances or interfere with the physiological effects of neurotransmitters and hormones. The advantage of this approach is the ability to make use of structural, pharmacological, physiological and biochemical information available for the target system; its disadvantage is that very little reliable experimental data is available concerning the actions of xenobiotics on such well defined systems. Additional complications are that metabolites of

the xenobiotics may constitute the active (and toxic) species while little is known about the metabolic fate of the xenobiotics, and that "non-specific" actions of these agents may be important in producing the toxic effects. Such non-specific actions are based on those physicochemical properties of the agents that produce changes (e.g., of pH or viscosity) in the local environment in which the agents accumulate (e.g., cells, intercellular space, membranes). A comprehensive understanding of the structure-activity relationships (SAR) for the toxic actions of xenobiotics, which is essential for the ability to predict their toxicity, must therefore take into consideration the molecular determinants for both specific and non-specific actions. This makes it necessary to evaluate the potential for recognition of xenobiotics at specific targets including the receptors and the enzymes involved in cellular processes and metabolism; and, in parallel, to elucidate the molecular structural elements that determine the physicochemical properties responsible for the non-specific effects of the agents and their metabolites. While a mechanistic approach to this task is clearly preferable, detailed mechanistic information is lacking for most toxic substances. In these cases, physical and chemical properties of the xenobiotic molecules can be used as parameters in a comparative correlative approach to the prediction of activities of untested chemicals on the basis of known properties of compounds that have been tested.

Correlative approaches must be based on the use of reliable physical properties as parameters for the comparison of different molecules. Because the basic assumption of SAR studies is that the action of exogenous compounds on biological targets depends on the same molecular properties that determine chemical interactions and reactions, the properties used in such correlations must be those that determine the chemical reactivity of the compounds. Taken together, such properties constitute the molecular reactivity characteristics of the compounds. The use of such reactivity characteristics as predictors of toxicity presents several advantages: 1) results can be incorporated directly in the analysis and prediction of toxicity because any existing information about mechanisms of action of a given class of compounds can be expressed in terms of such reactivity characteristics; 2) physical and chemical properties that determine the reactivity of the molecules can be used in correlative approaches with or without implication of specific mechanisms; 3) if based on reactivity characteristics, the resulting correlations can be used directly to provide insight into putative mechanisms of action.

The insight that stems from such a physicochemical basis provides some assurance that the correlations are not fortuitous and can therefore be used to assess the activity of xenobiotics that do not belong to the initial set of molecules for which the correlation was established. It follows, therefore,

that much attention must be given to the selection of properties that can adequately describe the reactivity characteristics [2,3].

Because the molecular properties that are directly responsible for the molecular interactions leading to the biological effect are encoded in the entire molecular structure, the description of the chemical reactivity characteristics must also rest on a representation of the properties of the entire molecule. This consideration reveals one of the major drawbacks of the approaches in which structure-activity relationships are studied on the basis of parameters representing contributions from certain structural components ("functional groups", "substituents"). These parameters used in "quantitative SAR" (QSAR) may fail to provide a mechanistic explanation of the observed correlation between molecular structure and biological activity for they seldom constitute an adequate representation of molecular properties [3]. Useful predictive criteria for biological activity should therefore be constructed from molecular properties derived from the entire molecular structure.

As was shown to be the case with the prediction of pharmacological properties of drugs [2,4], and for the study of enzymatic processes [5-7], the use of determinants of molecular reactivity as criteria for explaining and predicting toxicity rests on the causal link between molecular structure, molecular properties and the mechanisms responsible for the molecular interactions in the biological systems [7]. This emphasizes the importance of criteria that are solidly based on the principles of theoretical chemistry.

We have recently reviewed the structural and functional considerations leading to the evaluation of molecular properties that determine reactivity and must therefore determine the biological actions of xenobiotics [1]. We analyzed the methods underlying the correlations of activity with structural characteristics such as molecular size, shape, topology and conformation, with elements of molecular reactivity such as the electron density distribution and its moments (e.g., dipole, quadrupole), and with the molecular electrostatic potentials (MEP). The essential features of these methods are briefly presented in two sections following below, and are then illustrated in an application to the comparison between a toxic and a non-toxic pyridine derivative.

REACTIVITY CRITERIA BASED ON STRUCTURAL CONSIDERATIONS

Structural Comparisons

The consideration of structural parameters can serve as a first lead in the evaluation of the potential for toxic activity of xenobiotics. Structural similarity between a molecule with known properties and another molecule can

provide a basis for prediction of the other molecule's properties because the physicochemical properties encoded in the similar structures of the molecules, may themselves be similar. It is therefore necessary to use methods of structural comparison that will quantify the degree of similarity and relate it to the properties responsible for molecular reactivity. Some methods of structural superposition that can be used to perform such quantitative comparisons were reviewed [1]. The methods were classified into those that perform rigid comparisons [8-11] and those that allow for conformational rearrangements to optimize the superpositions [12-15]. Both approaches are based on the identification of the equivalent atoms in the compared molecules and the derivation of a rotation-translation matrix to optimize their superposition. In the flexible superposition procedures [12-15], free rotations around the bonds are permitted during the superposition process. An advantage of the latter approach is that it can identify the similarity between non-contiguously linked substructures of molecules, thus allowing for a comparison of the substructures that might be essential for interaction with biological targets.

A method of flexible structural comparison that does not require direct superposition of the molecules is based on the "distance geometry" algorithm [15] in which distance constraints are imposed on interatomic distances in one molecule to match those in another. In addition to considerable efficiency compared to the conformational search methods (e.g., see [12]), this approach also offers the capability to incorporate criteria other than structural similarity into the comparison of two molecules. Such criteria, based on physicochemical molecular properties, are useful in comparisons of molecules with dissimilar structures, but similar biological activities; notably, they are conducive to mechanistic inferences (see above).

Other methods for structural comparison that do not require direct superposition are based on the Distance Matrix Analysis [16,17] in which the three-dimensional molecular structure is represented by a square matrix of order N , the number of atoms. The elements of the distance matrix are the distances between atom pairs. The use of this method illustrates its power to identify molecular structural homology [18,19] and conformational perturbations ascribable either to changes in substructure conformation (e.g., see examples in [1, 18]) or to the spatial orientation of the substructures (e.g., see [1,19, 20]).

Exposed Surface Area and Surface Accessibility

The surface area presented by a molecule for its interaction with any reagent, and especially the steric accessibility of its most reactive sites, constitute key elements of molecular reactivity [21]. The definition, calcu-

lation and display of such surfaces is therefore necessary for the analysis of biological activity of xenobiotics, and provide additional parameters for the comparative prediction of toxicity [1].

A direct definition of the surface is obtained from the spheres surrounding the atoms of a molecule at the atomic van der Waals radii. Another approach defines the molecular surface as that accessible to a reagent, specifically a water molecule [22,23]. In practice, the reagent is often replaced by a sampling probe chosen as a sphere with an appropriate radius. The choice of size of the sampling probe and of the distance at which it is allowed to approach determine the shape and reactivity characteristics of the surface [1].

An efficient method for the calculation of total molecular surface areas, volumes, and atom accessibilities was also described recently [1]. Briefly, it is based on a Monte Carlo simulation of space filling within a box of enclosure containing the molecule. The volume of the box is computed by enumeration of the number of points generated randomly at a certain density (e.g. 50 points per $\text{\AA}^3$) within the box. Molecular volumes are evaluated by enumeration of such points lying within the van der Waals surface defined by the spheres surrounding the atoms at the corresponding van der Waals radii. The exposed surface area of an atom is computed in an analogous manner from the enumeration of points randomly generated at uniform density at the fixed van der Waals radius for each atom. The ratio of the number of such points that are at a van der Waals distance from only one atom to the total number of points defines the available surface area for that atom. As shown in the illustration given in a section below, this concept can be generalized to yield information on the "reactivity surface" of an atom, by recalculating such accessible surface at different distances from the nucleus. This surface accessibility as a function of distance is shown in that illustration to yield insight into the effect that intramolecular steric hindrance has on reactivity.

REACTIVITY CRITERIA BASED ON THE MOLECULAR ELECTROSTATIC POTENTIAL

The chemically reactive sites in molecules, and the long-range intermolecular forces they generate, constitute the basis for the calculation of molecular properties relevant to interactions pertaining to biological recognition [1-5]. For this reason, molecular electrostatic potentials (MEP) have been widely used, and with considerable success, in the prediction of biological activity [2,24]. Because the MEP is a rigorously defined quantity obtainable directly from the molecular charge distribution [24], a map of the MEP constitutes a faithful representation of the reactive regions created by the molecule in its surroundings. Regions of negative potential are attractive to positive

species or positively charged regions of approaching reagents, whereas regions of positive potential are repulsive to positive and attractive to negatively charged reagents. This offers a description of the localization and shape of the reactive regions of a molecule, a pattern that characterizes the requirements for optimal electrostatic interaction with the molecule and hence is predictive of its reactivity towards various reagents [2]. Because such a description transcends atom-by-atom analysis of the similarity of two molecules it offers a basis for the comparison of their reactivity without reference to their atomic structure, a clear advantage in the analysis of toxic properties in the large variety of chemical classes presented by the xenobiotics of interest [1]. Thus, characteristics of MEP can be compared among active and inactive species to infer on the properties that are relevant to activity. An illustration of this approach is given below for the case of toxic and non-toxic pyridine derivatives.

The comparison of the reactivity properties of the pyridine derivatives presented below, exemplifies the variety of choices available for the calculation and representation of the molecular properties, e.g., the MEP [1]. In particular, the calculation of the MEP on the surface surrounding the molecule at a distance proportional to the van der Waals radius of the atoms (i.e., at various multiples of the van der Waals radius) provides a representation that is directly relevant to the interaction of the molecule with an approaching species, and implicitly considers the accessibility of each molecular region (see above).

The considerations raised by various methods for computing, displaying and analyzing the MEP on such molecular surfaces were discussed elsewhere [1]; some of the consequences are illustrated below, in the analysis of pyridine derivatives. Briefly, the analysis of MEP must consist of two major components: the identification of extrema (i.e., minima and maxima) on the surface on which the MEP is calculated, and the formulation of a characteristic pattern of extrema and other regions in the MEP map that represent a "fingerprint" of the molecules recognized by their biological target [25]. Such spatial relationships between extrema in the MEP map are determined by the molecular structure and charge distribution. They also define the geometrical constraints that determine the orientation of the molecule in nearly homogeneous electrostatic fields or highly polar regions [1-4] such as a xenobiotic would encounter at relatively large distances from charged centers. Such elements are evident in the maps of MEP calculated for the pyridine derivatives discussed below, but a reactivity map relevant to biological recognition of these agents has not yet emerged.

REACTIVITY CHARACTERISTICS OF PYRIDINE DERIVATIVES RELATED TO THEIR TOXICITY

The application of some theoretical methods to the analysis of molecular determinants for toxicity is illustrated below for pyridine derivatives.

Toxic effects of pyridine derivatives have been demonstrated in various animal species [26-31]. Metabolic N-oxidation was demonstrated both in vivo and in vitro for toxic compounds including nicotinamide and nicotinic acid [32-35], as well as for pyridines with a variety of substituents at the 3 position ($-\text{CH}_3$, $-\text{C}_2\text{H}_5$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{COCH}_3$, $-\text{CN}$, $-\text{CON}$, $-(\text{C}_2\text{H}_5)_2$) which were shown to undergo N-oxidation by microsomal preparations in vitro [36]. These findings lead to the suggestion that the N-oxides are produced by a cytochrome P-450 type enzyme and that the metabolites could be responsible for the toxic effects [37]. Interestingly, 2,6-dimethylpyridine (DMP) appears to be non-toxic and the N-oxide does not seem to be a major metabolite. Rather, an examination of the various metabolites of alkylpyridines [38] revealed that the major metabolite of DMP is 2-methyl-6-carboxylic acid. Such a difference in the properties of compounds in the pyridine series suggests that the identification of molecular determinants for their toxicity may be related to the requirements for biotransformation into their N-oxide form. Notably, DMP forms the N-oxide derivatives under non-enzymatic conditions: N-oxidation by perbenzoic acid is nearly twice as fast for DMP as for pyridine [39]. The theoretical methods described above were therefore used to evaluate the basis for the differences as well as the similarities in the reactivity properties of DMP and pyridine.

Comparison of the molecular electrostatic potentials of DMP and pyridine calculated in the planes of the molecules (Fig. 1) shows the similarity of the main reactivity properties determined by the ring nitrogen. The highly nucleophilic character of the region near the nitrogen is expressed in the strong localized negative potential. This reactivity property is in agreement with the accepted mechanism for N-oxidation [40]. Moreover, the larger absolute value of the minimum in DMP is consistent with the data showing faster N-oxidation kinetics in DMP than in pyridine [40,41]. However, the region of negative potential is clearly narrower in DMP than in pyridine - a consequence of the positive potential generated by the neighboring methyl groups. As discussed below, this narrowing limits the accessibility of a positively charged species and is an energetic expression of the geometrical concept of "steric hindrance".

The nature of the spatial confinement produced by the methyl substituents is evident from a comparison of the molecular surfaces calculated at a distance of one van der Waals radius from each atomic center. A significant difference between the molecules becomes apparent from a comparison of these molecular

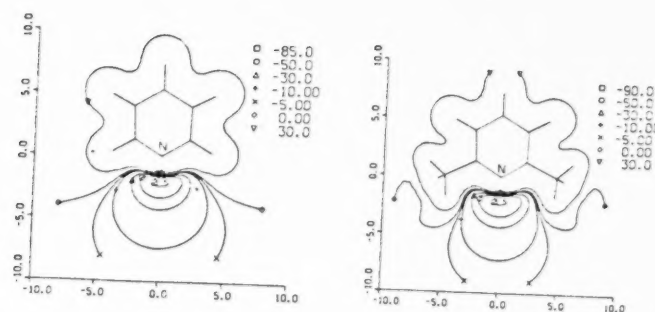


Fig. 1. Molecular electrostatic potentials of pyridine (left) and 2,6-dimethylpyridine (right) calculated in the planes of the molecules. Values in Kcal/mole.

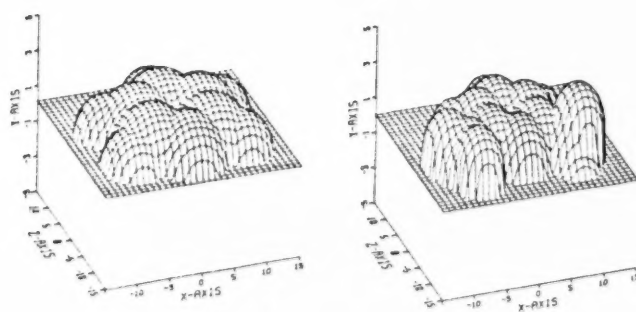


Fig. 2. Molecular surfaces of pyridine (2A, left) and 2,6-dimethyl pyridine (2B, right) viewed from the Y direction above the molecular planes and calculated at a distance of one van der Waals radius from each atomic center. Due to symmetry, only one half of the molecular surface is shown. Nitrogens are shaded.

surfaces (Fig. 2). In pyridine (Fig. 2A), the nitrogen should be easily accessible at a distance corresponding to the nearest approach of the envelope of a reagent molecule (i.e., at a distance of 1.5 times the van der Waals radius - see below). In contrast, the approach to the nitrogen is obstructed in DMP (Fig. 2B) by the bulky methyl groups on either side. This obstruction is best seen from the vantage point of an approaching species, as shown in Fig. 3. The molecular surface surrounding the "edge" of the molecule as seen when approaching directly towards the nitrogen (i.e., the Z direction) is compared for pyridine (Fig. 3A) and DMP (Fig. 3B). Clearly, the reactive portion of the electron density of the nitrogen is protruding in Fig. 3A, and is recessed in Fig. 3B, thus illustrating the "steric hindrance" produced by the neighboring methyl group.

Using an extension of our algorithm for the calculation of exposed surface area [1] we can simulate the conditions of the steric interaction at various distances. The calculation of the exposed surface area of an atom is based on spheres surrounding each atom at the appropriate van der Waals radius. As described in a section above, a set of randomly distributed points is generated on the surface of each sphere, with a density of 50 points/Å². The exposed surface area is then defined as the surface area of the sphere multiplied by the ratio of the number of points that appear on an area not within the van der Waals radius of any other atom, to the total number of points on the surface of the sphere. To simulate the "accessibility" of the atom at larger distances, as an indicator of long range recognition of the properties of that atom, we augmented the van der Waals radius of the target atom by an expansion factor, and recalculated the accessibility parameter. Table 1 below presents a comparison of the accessibility to the nitrogen in pyridine and DMP, for several values of the expansion factor. Percent exposure indicates the proportion of the sphere area that is unencumbered by neighboring atoms.

TABLE 1 Exposed Surface Accessible at Various Distances from the Nitrogen in Pyridine and DMP

Expansion Factor:	1.0	1.5	2.0	2.5	3.0	3.5
Distance from nitrogen (Å)	1.5	2.25	3.0	3.75	4.5	5.25
Exposed area in pyridine (Å ²)	0.9	16.3	59.8	134	229	331
Exposed area in DMP (Å ²)	0.9	14.5	48.0	95	181	331
% Exposed area in pyridine	3	26	53	76	87	96
% Exposed area in DMP	3	23	42	51	71	96

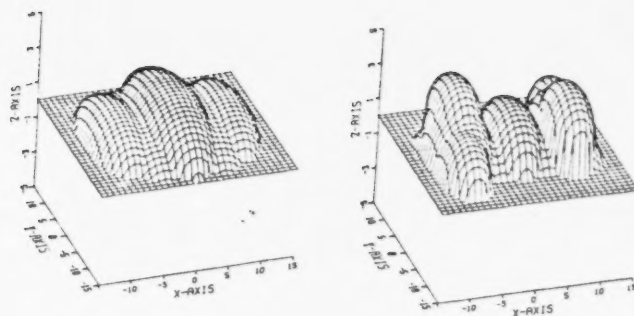


Fig. 3 .Molecular surfaces of pyridine (3A, left) and 2,6-dimethyl pyridine (3B, right) viewed from the Z direction facing the nitrogen atoms and calculated at a distance of 1.5 times the value of the van der Waals radius from each atomic center. Nitrogens are shaded.

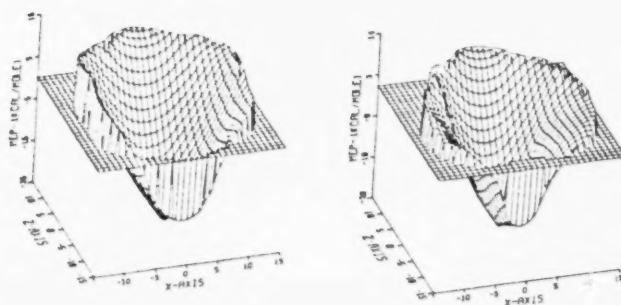


Fig. 4 .Three dimensional representation of the molecular electrostatic potential generated by pyridine (4A, left) and 2,6-dimethyl pyridine (4B, right) on a molecular surface viewed from the Y direction (Fig. 2) at a distance of two times the van der Waals radius from each atomic center.

Evident from the results in Table 1 is the considerable difference in the accessibility of the nitrogen at distances ranging from 3 to 4.5 Å from the nucleus. Interactions at these distances are crucial at the initial stages of molecular recognition and bond formation and the accessibility differences should be determinant for the differences in the reactivity properties of the compounds. This conclusion is reinforced by the results from a calculation of the MEP of the molecules on a molecular surface placed at 2 times the van der Waals radius of the atoms.

A three-dimensional representation of the electrostatic reactivity property, the MEP, calculated on such molecular surfaces surrounding pyridine and DMP is given in Fig 4. As was also the case for the MEP calculated in the plane of the molecules, the strongest feature is the negative potential generated in the vicinity of the nitrogen. The effect of the 2,6-substituents becomes evident from a comparison of the potentials of pyridine (Fig. 4A) and DMP (Fig. 4B): the potential-well generated by DMP is deeper but narrower than that of pyridine, and is surrounded by higher values of positive potential. This picture reflects the constrained, hindered nature of an electrostatically driven reaction at the nitrogen, but the enhanced stabilization provided by the increased localization of electron density due to the substituent effects.

The comparative analysis of the reactivity properties of DMP and pyridine reveals the structural and electronic elements responsible for the differences in the formation of the N-oxide derivatives of these molecules. The effects of the substituents flanking the ring nitrogen on the electronic structure of the molecule have enhanced the reactivity of this nucleophilic center but have markedly reduced its accessibility.

These findings offer inferences on the requirements for enzymatic N-oxidation and constitute new criteria against which pyridine derivatives can be tested to predict N-oxidation and hence, the production of a species with putative toxic effects.

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